METHODS OF STUDYING PERMEABILITY OF PROTOPLASM TO SALTS

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Investigation of the permeability of protoplasm to electrolytes has led to many apparent conflicts between evidence secured by different methods and between the theoretical conclusions based thereon. An intensive study of the evidence, and of the methods themselves, has shown that these apparent conflicts are in large measure due to an imperfect understanding of the limitations of the methods or to unwarranted assumptions as to the nature and reactions of living matter. It is therefore of interest to consider critically the methods heretofore employed in the study of permeability in order to determine which of these methods may be considered most reliable, and thus to acquire a broader understanding of the problem, and to lay the foundation for further investigation.

The methods employed in the investigation of the permeability of protoplasm to electrolytes fall into 4 general categories, in which the criteria employed are: (1) chemical analysis of tissue extracts or of solutions bathing the tissues, (2) visible changes within the cell, (3) turgidity of cells or tissues, and (4) electrical conductivity of tissues or of masses of cells. To these may be added a diffusion method, which will be described by the writer in a subsequent paper.

Chemical analysis

ANALYSIS OF TISSUES OR TISSUE EXTRACTS.—Perhaps the earliest employment of a method for the detection of an inorganic salt within a living cell which had previously been bathed by a solution of that salt was that by JANSE (16). Filaments of a species of *Spirogyra* were caused to burst in distilled water containing diphenylamine. Cells which had been bathed in a potassium nitrate solution for some hours previous to testing gave a strong positive reaction in the extruded cell material at the moment of bursting, while those briefly immersed in the potassium nitrate solution, and

then tested, gave no reaction. This method, although positive, can yield only qualitative data.

As a method for the investigation of protoplasmic permeability, quantitative analysis of tissue extracts involves several important sources of error, among which may be mentioned the presence of salts in the intercellular spaces and in the cell walls, where they may be held in solution or by adsorption, variations of the concentration and constitution of expressed juices dependent on the pressure used in extraction (cf. MAMELI 30), and, most serious of all, adsorption or chemical union of the salts within the cell. Thus, while aluminium ions might displace potassium ions in an adsorption compound, sodium ions might displace the potassium ions to a small extent only. In this way the free aluminium content of the cell would remain low, and the original rate of endosmosis of aluminium salts would be maintained, while that of sodium salts would steadily decrease as the free sodium content of the cell increased. A similar effect might be produced by the formation of hydrates of aluminium and sodium; the former being insoluble would form a precipitate, while the latter would remain in solution.[1] The relative permeability of the tissues to different salts would then be made to appear other than it actually was. The last error also affects methods involving the amount of salt taken by tissues from a solution. Conclusions as to the permeability of the plasma membrane, therefore, cannot be safely based upon data furnished by experiments of this type, such as those of NATHANSOHN (38, pp. 453 ff.), PANTANELLI (51), DE RUFZ DE LAVISON (55, 56), COLIN and DE RUFZ DE LAVISON (4, 5), MEURER (36), and many others.

PAINE (50), using these methods, drew the conclusion that yeast cells are wholly impermeable to inorganic salts.[2] He found a slight absorption of these salts by the yeast cells, but attributed it to adsorption of the salts in the cell walls. Irrespective of the validity of his interpretation, there is no evidence that it is applicable to plants in general.

[1] Precipitates have been observed to form in living cells when they are treated with aluminium salts, but the nature of these precipitates has not been ascertained.

[2] Aqueous solutions of NaCl, $(NH_4)_2SO_4$, Na_3AsO_4, Na_2HPO_4, and sodium hexose phosphate were used.

ANALYSIS OF SOLUTIONS BATHING THE TISSUES.—The method of analysis may also be applied to the diffusion from living cells ("exosmosis") of substances normally present in the cells and retained by the impermeability of the protoplasm (they may accumulate in the cell walls of terrestrial plants in quantities sufficient to maintain a condition of equilibrium with the solution inside the cell, and may diffuse out when the cells are placed in water). Under certain conditions these substances may be made to diffuse from the cells in appreciable quantities. The experiments of WÄCHTER (**69**) on the exosmosis of sugar from onion bulb scales seemed to indicate that this exosmosis was inhibited by various salts. In the light of more recent evidence it seems possible that this was due to antagonization of traces of toxic salts in the "Leitungs-Wasser" which he used.

Other experiments have dealt with the absorption of salts from the solution as well as with exosmosis. The results of the experiments of TRUE (**63**), TRUE and BARTLETT (**64, 65, 66**), and MERRILL (**34, 35**), like those of WÄCHTER, were visible only after several hours, and the intervening effects upon permeability could not be determined. There was also opportunity for "regulative processes" and other complications to influence the absorption of salts to a marked extent during this interval, and a probability that some of the external cells would be killed and would give off their contained solutes to the surrounding solution. It is quite probable that these effects are of importance in experiments of such long duration as those of the investigators mentioned. The most serious objection to using the analysis of the solution as a criterion of permeability is that the method does not distinguish between permeability and absorption. These two things have little to do with each other. If the absorbed substance is trapped within the cell (by precipitation, or by a chemical change preventing it from diffusing out), it will continue to diffuse in, while a substance which is not trapped will soon stop diffusing in. Hence we see that absorption is no criterion of permeability, although it is so used by many investigators. The absorption of a substance may be great when permeability is small, and vice versa. The same objection applies to some extent to the use of exosmosis as a criterion, since

increased exosmosis may be due, not to increased permeability, but to increased production within the cell of the substance which diffuses out.

Visible changes within the cell

This method, although sometimes valuable in the investigation of the penetration of substances like the alkaloids which form intra-vitam precipitates, and acids and alkalies which cause color changes of pigments or intra-vitam stains, has found little application in the study of the penetration of inorganic salts.

OSTERHOUT (39) showed that crystals of calcium oxalate form in the root hairs of seedlings of *Dianthus barbatus* (previously grown in distilled water) within a few hours after their immersion in dilute solutions of calcium salts, and the subsequent normal growth of the cells proved that they were not injured. ENDLER (7) followed microscopically the entrance of intra-vitam stains (neutral red and methylene blue) into various plant cells under the influence of various kations. He also investigated the rate of disappearance of the dyes from stained cells, living and dead. The experiments are extremely instructive, showing that at 24 or more hours the passage of dyes through the membrane was increased by kations in the following order: $Na < K < Mg < Ca < Al$. Aluminium formed an exception in that at very low concentrations the exit of dyes from the living cells was inhibited. This inhibition was not observed in the experiments with dead cells, where the influence of the kations was due only to their physical action on the colloidal dye tannate formed in the cells. This series is precisely what would be expected of experiments of long duration on the supposition that a temporary decrease in permeability was produced by all the polyvalent kations, and that this was followed by an increase. Extreme dilution would prolong the period of decreased permeability, and would account for the inhibition of exosmosis which ENDLER found to occur when extremely dilute solutions of aluminium salts were used.

The interesting experiments of LOEB (29) on the diffusion of neutral red through the egg membrane of a marine teleost fish (*Fundulus* sp.), a process which occurs readily in electrolyte solutions but very slowly in distilled water, are apparently concerned

with the permeability of a membrane considerably different from the plasma membrane. LOEB suggests the theory that the dye kation is held in the membrane in a combination with a colloidal anion, and that this combination is broken down by the anions of a surrounding salt solution. The behavior of the potassium ion is shown to be like that of the dye kation, at least in its initial stages. Similar processes may occur in the plasma membrane, but it is not possible to apply LOEB'S conclusions directly to the behavior of unspecialized protoplasm.

HARVEY (12) has studied the permeability of plant cells to alkalies by introducing an intra-vitam stain, neutral red, which turns yellow in the presence of alkalies.[3] It was found that ammonia and the amines penetrated living and dead tissues with equal and very great rapidity, while the strong bases, although penetrating dead cells with great rapidity, required much longer to penetrate living cells. It seems possible that penetration of bases at the concentration used (0.025 N) was due to injury of the cells.

Turgidity of cells or tissues

The typical living cell behaves toward osmotically active solutions as though it were surrounded by an elastic semipermeable membrane. In view of the widespread confusion regarding the osmotic relations of living cells, it seems necessary to analyze, in so far as the present imperfect state of our knowledge of the physical laws governing osmotic phenomena will allow, the behavior of a cell which acts as a simple osmometer. It will then be possible to judge more accurately the value of the data furnished by the many methods based upon the study of the osmotic relations of living cells. Such a typical cell may be pictured as a body of solution surrounded by an elastic membrane permeable to and bathed by the solvent (in this case water), and slightly if at all permeable to the contained solute. Water will enter such a cell until the internal hydrostatic pressure produced by the tension of the stretched membrane just overcomes the tendency of water to enter the cell. There will thus arise a condition of equilibrium which will be main-

[3] Neutral red changes color between H ion concentrations of 6×10^{-6} and 1×10^{-8} N.

tained unless there is either a change in the tendency of the water to enter, or a change in the tension of the membrane. The latter has not been shown to occur, but may be responsible for certain as yet unexplained phenomena observed in plasmolytic experiments. The former will be produced by alterations in the concentration of the solution bathing the cell, an increase in its concentration causing a loss of water from the cell, with consequent shrinkage, and a decrease causing an intake of water with accompanying increase in volume. These changes will proceed until a new equilibrium is established at which the internal osmotic pressure is again equal to that of the external solution plus the pressure produced by the tension of the membrane.

The rate at which the exchange of water will occur is a function of the difference in osmotic pressure between the intra- and extra-cellular solutions and of the permeability of the membrane to water. There appears to be a tendency among physiologists to confuse the effect of the rate of penetration of a solute on that of water (which is produced by the resultant progressive change in the osmotic gradient), with a hypothetical effect, independent of the osmotic gradient, produced by the simultaneous passage of both solute and solvent through the membrane. There is no physical justification for the latter assumption, and the two ideas should be carefully distinguished. The change in volume of the cell is the sum of the change in volume due to diffusion of water and that due to diffusion of the solute. In cases where the solute is a substance like alcohol, the latter factor may be of considerable importance; but protoplasm is in general so much less permeable to inorganic salts than to water that their diffusion may be neglected in so far as their volume is concerned.

The intra- and extra-cellular osmotic pressures are thus quickly equalized by passage of water through the membrane, and a state of equilibrium is reached, which, if the membrane is permeable to water only, is permanent. But if a diffusion of solute occurs, it will cause changes in osmotic pressure which will lead to further water exchange, and this process will proceed until the solute attains an equal concentration in both intra- and extra-cellular solutions, and a true equilibrium is thus established. The rate at

which these changes occur will depend upon the permeability of the protoplasm to the solute and upon the concentration gradient causing the diffusion of the solute.

If a living cell be placed in a fairly concentrated salt solution, the salt usually diffuses into the cell (a process known as "endosmosis"), while the sugars, to which a large part of the intracellular osmotic pressure was originally due, remain for the most part within the cell. Under these conditions the cell will increase in volume, until it reaches the same turgidity (that is, the same degree of distension due to the tendency of water to enter the cell) which it would have possessed had there been no salt at all present. The outward diffusion of salts or other substances from the cell ("exosmosis") is usually negligible, but it is always to be remembered that such a diffusion may be occurring simultaneously with the endosmosis. If a salt after entering the cell forms there osmotically inactive compounds, either by adsorption or by chemical combination, and does not at the same time cause the liberation of an osmotically equivalent amount of some other substance, the turgidity of the cell is less than would be expected, and there is a decrease in the apparent rate of penetration of the salt.

If a plant cell be placed in a solution which causes shrinkage, the cell wall will contract elastically for a certain distance, and will then suffer no further change in size; meanwhile the continued shrinkage of the protoplasm will cause it to retract from the cell wall. This separation of the protoplasm from the cell wall is known as plasmolysis. It was first observed by PRINGSHEIM (52) in 1854, and was ascribed by NÄGELI (37) to the impermeability of the protoplast to the plasmolyzing substance. In this process the protoplasm may tear away just inside the cell wall, leaving attached to it a thin layer of protoplasm to which the central mass remains for a time connected by fine threads (cf. BOWER 1, CHODAT and BOUBIER 3, HECHT 13, and KÜSTER 22). The process of plasmolysis may then subject the protoplasm to a very considerable mechanical injury, and it is quite probable that its subsequent permeability will not be the same as that of a protoplast which has not been subjected to plasmolysis. In animal cells and tissues, where no cell walls are present, and in tissues of plants when shrinkage is not

carried far enough to cause plasmolysis, we have a means of avoiding this objection. We may consider first those methods in which plasmolysis occurs.

METHODS INVOLVING PLASMOLYSIS

1. *Concentration Required to Produce Plasmolysis.*—DE VRIES (67) noticed that the concentration of a glycerine solution just concentrated enough to produce plasmolysis was higher than that expected from the calculated osmotic pressure of the solution. He attributed this to the penetration of glycerine into the cell.

On the assumption that an increase in the concentration of a given substance required to produce plasmolysis indicates an increase of permeability, LEPESCHKIN (23, 24, 25, 26) and TRÖNDLE (62) claim to have demonstrated an increase of the permeability of the protoplasm due to increased illumination; and ECKERSON (6) seeks the cause of the thermotropic curvatures of roots in an increase in permeability due to rise in temperature. By the same method KREHAN (20, 21) has studied the effect of potassium cyanide on the permeability of cells of *Tradescantia discolor*, the experiments seeming to indicate that dilute solutions (0.001 M) of potassium cyanide cause a temporary and reversible increase in permeability, and that this is followed by a decrease in permeability which begins simultaneously with loss of the reversibility.

OSTERHOUT (40) has shown that solutions of sodium and calcium chlorides, either of which alone is unable to produce plasmolysis (of cells of *Spirogyra* sp.), may cause rapid plasmolysis when mixed in such proportions that the ratio of sodium atoms to those of calcium is about 20 to 1. Since the normal permeability of the protoplasm is most nearly attained in the mixed solution, which is a partially balanced mixture, it would appear that the permeability of the protoplasm was abnormally high in one of the pure solutions. It is possible, however, to establish a different interpretation of this phenomenon; this will be considered in the light of evidence secured by other methods.

FLURI (9) found that aluminium salts so altered the protoplasm of certain plant cells as to make it impossible to plasmolyze them.

This alteration he supposes to be the production of complete permeability. SZÜCS (61) has since stated that the alteration consists of a hardening of the protoplasm, since centrifuging no longer displaces the cell contents. He also found the "hardening" to be temporary, and to be followed by "reliquefaction."

LEPESCHKIN (27) claims to determine with great accuracy, by a method based upon the difference in the osmotic pressures of isotonic plasmolyzing substances, the absolute rate of penetration of these substances. It is impossible to explain the method clearly and at the same time briefly, but its essential features are as follows: a comparison of the osmotic pressure of a saccharose solution which will just cause visible plasmolysis, with that of a glycerine solution which, following the saccharose, will cause no change in volume (as determined by LEPESCHKIN's criterion) shows the latter to be the higher. If we let μ represent a factor proportional to the permeability of the protoplasm to the glycerine, and assume that the protoplasm is impermeable to saccharose, then $\mu=\frac{C^{1}-C}{C^{1}}$, where C^{1} is the concentration of glycerine found to be isotonic with the saccharose solution, and C the concentration calculated to be isosmotic with the saccharose solution. For saccharose we may substitute any substance to which the protoplasm is supposed to be impermeable, and for glycerine any substance whose rate of penetration it is desired to measure.

This method would be exact provided the following assumptions were in accord with the facts: (1) the protoplasm is impermeable to the control substance (in this case saccharose); (2) neither of the substances used causes any alteration in the permeability of the protoplasm; (3) no exosmosis occurs. All these assumptions are rendered highly improbable by the evidence already secured by other methods, and additional evidence against their validity will be submitted by the writer in a subsequent paper.

LEPESCHKIN also appears to assume that there is an effect on the water equilibrium caused by the simultaneous diffusion of solvent and solute through a membrane, and independent of the progressive changes in the osmotic gradient thus arising. This assumption is, as has been previously pointed out, without physical

basis. The method of LEPESCHKIN is therefore of extremely doubtful value.

2. *Recovery from Plasmolysis.*—Recovery of plasmolyzed cells was first noted by KLEBS (17) in 1887, who found that glycerine was able to penetrate the plant cell. He was unable to detect recovery of cells plasmolyzed by solutions of potassium nitrate or sodium chloride. DE VRIES (68) obtained similar results at about the same time. JANSE (16), whose work has been quite generally overlooked, demonstrated the penetration of potassium nitrate, sodium chloride, and saccharose by observations on the recovery of plasmolyzed cells of the marine algae *Chaetomorpha aerea* and *Dictyota* sp., and *Spirogyra nitida*, *Tradescantia discolor*, and *Curcuma* sp. It was thus conclusively shown that at least some inorganic salts can penetrate living cells of many types of plants.

OVERTON (48) was unable to observe any cases of recovery of cells plasmolyzed by inorganic salts. He supposed this to be due to the insolubility of such salts in lipoid substances, which he supposed to constitute the plasma membrane. It has been pointed out by OSTERHOUT (41) that OVERTON in all probability overlooked the recovery of the cells which he used, confusing the subsequent "false plasmolysis," due to the injury of the cells, for a continuation of the true plasmolysis. OSTERHOUT showed that a great variety of salts penetrate and cause recovery. OSTERHOUT also showed that the rate of recovery of *Spirogyra* cells was more rapid when a salt of one of certain monovalent kations was used to produce plasmolysis than when a calcium salt was similarly used.[4] It was impossible to establish more than the most general quantitative relations in these experiments. Recently FITTING (8) has conducted an extensive series of investigations on the permeability of cells of *Tradescantia discolor* L'Heritier (*Rhoeo discolor* Hance). His data may be most easily understood if stated graphically. Comparable strips of epidermis were plasmolyzed in a series of solutions differing by equal increases in molecular concentration. If there was no difference in the rate of recovery, a curve in which the ordinates represented recovery time and the

[4] The solutions used in these experiments were in each case of the lowest concentration which would produce plasmolysis.

abscissae concentration of the plasmolyzing solution would be a straight line. It was found, however, that such a curve was concave to the axis of the ordinates. This indicated a decrease in the rate of recovery with time. FITTING considers this to establish the fact that such salts cause a progressive decrease in the permeability of the protoplasm. He considers the possibility that exosmosis might have occurred in his experiments, and cites experiments which supposedly show that all possible exosmosis had taken place during the preliminary 4–6 hours' exposure of the tissues to distilled water. There are serious discrepancies in his data, such as the fact that a solution of a higher osmotic pressure is required to produce plasmolysis in tissues from which all possible exosmosis is supposed to have taken place than is required to produce it in otherwise comparable tissues from which no exosmosis has occurred. It is probable that FITTING has some important variables in the method which he has employed, and since he has failed to investigate the effect of salts of monovalent kations on exosmosis, it is probable that the supposed decrease of endosmosis is in reality an increase of exosmosis, which would have the same effect on the rate of recovery. FITTING also states that the cells are wholly impermeable to salts of bivalent and trivalent kations, with the possible exception of strontium. This is in conflict with the experiments of OSTERHOUT.

METHODS NOT INVOLVING PLASMOLYSIS

In rapidly elongating plant tissues there is usually a very considerable pressure exerted by the protoplasts against the cell walls which confine them. If all the cell walls of the stem are thin and elastic, the whole stem will be kept in a stretched condition by this pressure. The presence of thick-walled cells, such as fibrovascular or epidermal cells, which do not yield to internal pressure, will, if they are symmetrically distributed, prevent this elongation of the tissue. If we cut such a stem or peduncle so that these two types of tissue are unsymmetrically distributed, the whole tissue will curl so that the elastic tissue forms the longer or convex side. The distention of the elastic tissues, and therefore the degree of curvature, will vary with the turgidity of the tissues. A hypertonic

solution will withdraw water from the cells, and consequently reduce the turgidity and the degree of curvature, while a hypotonic solution will have the opposite effect. The penetration of the protoplasm by a salt with whose solution such a tissue had come into osmotic equilibrium would lead to an increase in the turgidity, and hence in the curvature of the tissue. De Vries (67), in the investigation of the isotonic coefficients of various substances by this method, observed such a secondary increase in curvature. Such tissue curvatures have not since been used in quantitative researches on the permeability of the protoplasm. The writer, however, has found it possible to make use of this method for quantitative determinations of permeability (Brooks 1a).

Changes in the volume or weight of animal cells or tissues have been used by many investigators to determine the rate of penetration of electrolytes. Red blood corpuscles and striated muscle have been the most frequently used materials.[5] As an example of the former, the work of Kozawa (19) may be quoted. This investigator added to 1 cc. of corpuscles centrifuged from defibrinated blood of various mammals 2 cc. of various solutions of equal osmotic pressure (as judged by the freezing point depression). The corpuscles were again centrifuged after a time varying from 15 minutes to 23 hours, and the volume of the mass of corpuscles noted.[6] Increase of volume was considered to indicate penetration of the solute. Sodium salts were not observed to cause any increase in volume. Koeppe (18) made similar observations.

In some animals glucose appeared to penetrate; in others it did not. It was found to be impossible to influence the permeability to glucose by various agents, including certain inorganic salts. These conclusions agree with those obtained by the use of quantitative analytical methods, notably those of György (10), who was unable to influence the rate of penetration of glucose into red blood corpuscles by suspension of the corpuscles in buffer solutions of

[5] Changes in weight of whole organisms have been used in the study of osmotic relations. Cf. Overton (48), Quinton (53), and Henri and Lalou (15).

[6] The determination of volume changes in red blood corpuscles by centrifuging was first suggested by Hedin (14), and is known as the "haematocrit" method. Similar methods have been applied to other free cells such as leucocytes and spermatozoa. Cf. Hamburger (11).

various hydrogen ion concentrations, or in solutions containing Ca, Mg, Mn, oxalate, or SO_4 ions (cf. also MASING 31 and LOEB 28).

OVERTON (49) made successive determinations of the weight of sartorius muscles of the frog during treatment with various solutions. He reports that no increase in weight took place in a 0.7 per cent sodium chloride solution during a period of many hours; that isotonic solutions of the phosphate, tartrate, sulphate, ethyl sulphate, and acetate of potassium induced no change in weight during 50 hours. After a few hours an increase of weight occurred in solutions of potassium chloride, iodide, bromide, and nitrate, but OVERTON found these changes to be irreversible, and concludes that the normal muscle is impermeable to neutral salts. SIEBECK (57), on the other hand, finds that under proper conditions the increase in weight of kidney tissue in a pure isotonic solution of potassium chloride is reversible, and therefore considers that these cells are normally permeable to potassium chloride. In general the permeability of animal cells to neutral salts seems to be less, and more often characterized by selective peculiarities than that of plant cells. The red blood corpuscles, for example, may well be considered to be surrounded by a considerably specialized protoplasmic envelope.

The experiments of LOEB (29) on the permeability of fertilized *Fundulus* eggs to electrolytes are concerned with a peculiarly specialized envelope surrounding the embryo. This envelope is characterized by an exceedingly small permeability to salts. Thus an embryo 4–14 days old within the egg membrane survives 3 days of exposure to a solution (50 cc. 3 M NaCl+1 cc. 10/8 M $CaCl_2$) which is almost instantly fatal to the newly hatched fish. As has previously been noted, generalizations as to the permeability of protoplasm cannot be made from data furnished by experiments on such a membrane, and a more extended discussion of the results of these experiments would not be profitable here.

Electrical conductivity of tissues or of masses of cells

The conduction of electrical current by a solution involves the passage through the solution of electrically charged atoms of

some substance. These charged atoms, known as ions, are not created by the electrical conditions imposed, but already exist in all solutions capable of conducting a current. The rate at which the current will be conducted by the ions of a given salt will depend upon two factors, the potential gradient and the frictional or other resistance to the migration of the ions. If the potential gradient be kept constant, we may follow fluctuations in the last factor by a measurement of the current, or by a direct measurement of the electrical resistance. If, therefore, we force the current to pass through living protoplasm in a solution, the resistance offered by the protoplasm to the passage of the ions will measure its permeability to the ions in question (the permeability may be regarded as varying inversely as the resistance). By the use of alternating currents of rather high frequency we avoid large effects due to accumulation of ions at surfaces impermeable to them.[7]

A method of this type was independently employed at about the same time by RÓTH (54), BUGARSKY and TANGL (2), and STEWART (58), who found that the conductivity of blood serum was greater than that of blood itself, and that the resistance rose rapidly with increase of the proportion of corpuscles to serum. The blood corpuscles seemed to be slightly or not at all permeable to the electrolytes of blood. The conductivity of the suspension of corpuscles was shown to be increased by haemolytic agents, the corpuscles then being permeable to salts (cf. WOELFEL 70, also STEWART 58, 59). MCCLENDON has also attempted to study the changes in permeability of sea urchin eggs during fertilization (32) and of muscles in tetanus (33). The evidence from his experiments on sea urchin eggs agrees with that of HARVEY (12), previously mentioned, but difficulties in technique which MCCLENDON found it impossible to avoid make the data of these experiments exceedingly unreliable.

The experiments of OSTERHOUT (42–47) on the conductivity of tissue of the marine alga *Laminaria* have shown the important fact that the permeability of living protoplasm is altered by salts in pure and mixed solutions in a manner characteristic of the ionic constitu-

[7] The small capacity effect will be proportional to the resistance, so that no relative error is thereby introduced.

tion of the solution. The kations are of particular importance. All monovalent kations (excepting the hydrogen ion) produce only an increase in permeability of the protoplasm. This increase, reversible in its first stages, finally leads to death and complete permeability. Bivalent and trivalent kations and the hydrogen ion cause a temporary and reversible decrease of permeability which is followed or superseded by an increase which is irreversible and leads to death of the cells. In a balanced solution such as sea water the resistance remains constant provided the laboratory conditions are such as to maintain the full vitality of the tissue. We have here a method of determining quantitatively the permeability of the protoplasm at any instant, and the data secured demonstrate the extreme importance of progressive changes in the permeability of protoplasm. It would be possible to imagine that the passage of an electrical current through the tissues was responsible, at least in some measure, for the observed changes in permeability. It would be of advantage, therefore, to check the results of OSTERHOUT'S method by the use of some method entirely free from this possible objection. The method is also applicable to certain types of tissue only, and it is desirable to extend to other types of plants the principles derived by the application of this method.

The plasmolytic experiments of OSTERHOUT (40) may be explained in the light of the experiments by the conductivity method in the following manner. During the time required to produce plasmolysis the permeability has considerably increased in the sodium chloride solution and somewhat decreased in the calcium chloride solution. In that time much more sodium chloride has penetrated the cell, therefore, than of the salts of the mixed solution in which the permeability remains normal, and these again more than the calcium chloride, and the osmotic gradients have changed accordingly. The osmotic pressures of the solutions which will produce visible plasmolysis will then have suffered an increase over the actually isosmotic solutions, and in this order: calcium chloride very little, the mixed solution slightly more, and sodium chloride considerably more. If we now mix a large amount of sodium chloride of a concentration just insufficient to produce plasmolysis with a small amount of a similar calcium chloride

solution, the resulting osmotic pressure will be considerably above that of a similar mixed solution (that is, one just insufficient to cause plasmolysis), and plasmolysis will result.

Summary

From a consideration of the methods heretofore used in the study of permeability it would appear that the steps most essential to further progress toward the solution of the problem are: (1) a thorough analysis of the various disturbing factors in the methods involving chemical determinations and the satisfactory interpretation of the results secured by such methods; (2) the same type of analysis of the methods depending on turgor, with special reference to the possible effect of exosmosis; and (3) the establishment of methods of determining progressive changes in permeability without the various disadvantages of the other methods.

The writer hopes to show in subsequent papers that the diffusion method, which he has devised, answers these requirements, and that it is also possible to interpret satisfactorily the data obtained by certain methods dependent upon the use of turgor as a criterion.

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